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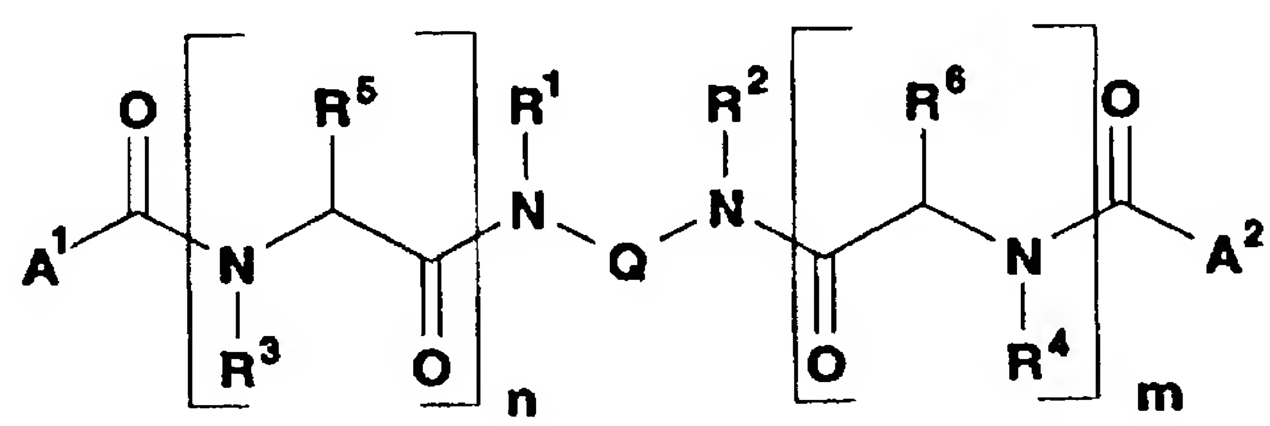
(54) Title: HEMOREGULATORY COMPOUNDS

(57) Abstract

Novel compounds of general formula (I) which have hemoregulatory activities and can be used to stimulate haematopoiesis and for the treatment of viral, fungal and bacterial infectious diseases.



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HEMOREGULATORY COMPOUNDS

Field of the Invention

The present invention relates to novel compounds which have hemoregulatory activities and can be used to stimulate haematopoiesis and for the treatment of viral, fungal and bacterial infectious diseases.

Background of the Invention

The haematopoietic system is a life-long cell renewal process whereby a defined stem cell population gives rise to a larger population of mature, differentiated blood cells (Dexter TM. Stem cells in normal growth and disease, Br Med J 1987; 195:1192-1194) of at least nine different cell lineages (erythrocytes, platelets, eosinophils, basophils, neutrophils, monocytes/macrophages, osteoclastes and lymphocytes) (Metcalf D. The Molecular Control of Blood Cells, 1988; Harvard University Press, Cambridge, MA). The stem cells are also ultimately responsible for regenerating the bone marrow following treatment with cytotoxic agents or following bone marrow transplantation.

The major dose-limiting toxicities of most standard anti-neoplastic drugs are related to bone marrow suppression, which if severe and prolonged, can give rise to life-threatening infectious and haemorrhagic complications. Myelosuppression is predictable and has been reported to be dose-limiting in greater than 50% of single-agent Phase I trials cytotoxic compounds (Merrouche Y, Catimel G, Clavel M. Haematopoietic growth factors and chemoprotectants; should we move toward a two-step process for phase I trials in oncology? Ann Oncol 1993; 4:471-474). The risk of infection is directly related to the degree of myelosuppression as measured by the severity and duration of neutropenia (Brody GP, Buckley M, Sathe YS, Freireich EJ. Quantitative relationship between circulating leukocytes and infections with acute leukemia. Ann In Med 1965; 64:328-334).

The control of haematopoiesis involves the interplay of a variety of cytokines and growth factors during various stages of the haematopoietic cascade, including early pluripotent stem cells and mature circulating effector cells. These regulatory molecules include granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage stimulating factor (GM-CSF), macrophage-colony stimulating factor (M-CSF), and a variety of interleukines which have overlapping, additive and synergistic actions which play major roles in host defence. Mechanistically, this is accomplished by enhancing the production of granulocytes and macrophages, as well as by the activation of effector cell functions (Moore MAS. Haematopoietic growth factor interactions: in vitro and in vivo preclinical evaluation. Cancer Surveys 1990; 9:7-80). These co-ordinated activities support optimal host defences which are necessary for fighting bacterial, viral and fungal infections.

Strategies to prevent or reduce the severity of neutropenia and myelotoxicity include the use of haematopoietic growth factors and/or other haematopoietic cytokines. Such treatments are becoming common practice, in that they offer the potential of increased doses of cytotoxic agents that may improve the therapeutic efficacy if antineoplastic agents, and reduce the morbidity associated with their use (Steward WP. Granulocyte and granulocyte-macrophage colony stimulating factors, Lancet 1993; 342:153-157). Clinical studies have demonstrated the G-, GM- and/or M-CSF may reduce the duration of neutropenia, accelerate myeloid recovery and reduce neutropenia-associated infections and other infectious complications in patients with malignancies who are receiving cytotoxic chemotherapy or in high infectious-risk patients following bone marrow transplantation (Steward WP. Granulocyte and granulocyte-macrophage colony stimulating factors, Lancet 1993; 342:153-157 and Munn DH, Cheung NKV. Preclinical and clinical studies of macrophage colony-stimulating factor. Semin Oncol 1992; 19:395-407).

We have now found certain novel compounds which have a stimulative effect on myelopoietic cells and are useful in the treatment and prevention of viral, fungal and bacterial diseases.

Summary of the Invention

This invention comprises compounds, hereinafter represented as Formula (I), which have hemoregulatory activities and can be used to stimulate haematopoiesis and in the prevention and treatment of bacterial, viral and fungal diseases.
These compounds are useful in the restoration of leukocytes in patients with lowered cell counts resulting from a variety of clinical situations, such as surgical induced myelosuppression, AIDS, ARDS, congenital myelodysplacis, bone marrow and organ transplants; in the protection of patients with leukopenia from infection; in the treatment of severely burned patients and in the amelioration of the myelosuppression observed with some cell-cycle specific antiviral agents and in the treatment of infections in patients who have had bone marrow transplants, especially those with graft versus host disease, in the treatment of tuberculosis and in the treatment of fevers of unknown origin in humans and animals. The compounds are also useful in the treatment and prevention of viral, fungal and bacterial diseases, particularly Candida, Herpes and hepatitis in both immunosuppressed and "normal" subjects.

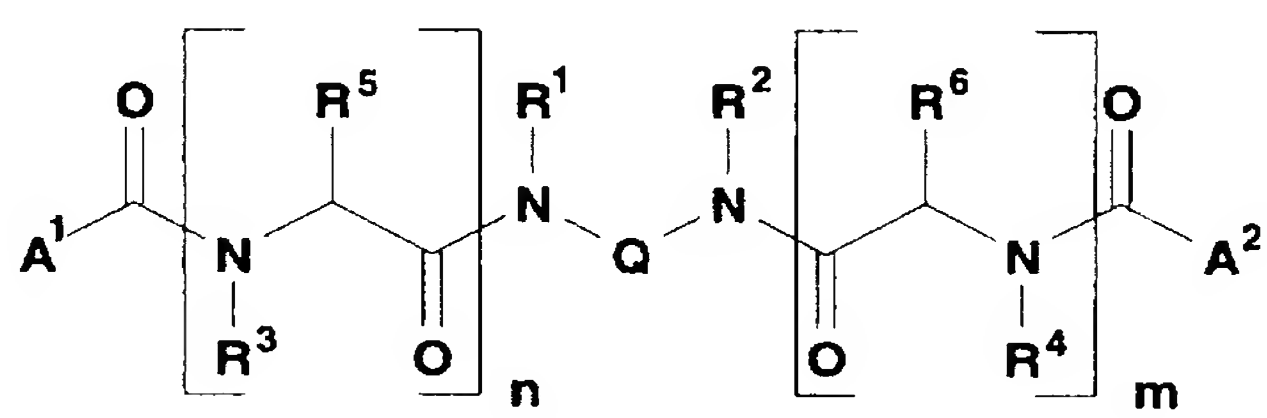
These compounds may also be used in combination with the monomers of co-pending U.S. Application No. 07/799,465 and U.S. Patent No. 4,499,081, incorporated by reference herein, to provide alternating peaks of high and low activity in the bone marrow cells, thus augmenting the natural circadian rhythm of haematopoiesis. In this way, cytostatic therapy can be given at periods of low bone marrow activity, thus reducing the risk of bone marrow damage, while regeneration will be promoted by the succeeding peak of activity. This invention is also a

pharmaceutical composition, which comprises a compound of Formula (I) and a pharmaceutically acceptable carrier.

This invention further constitutes a method for stimulating the myelopoietic system of an animal, including humans, which comprises administering to an animal in need thereof, an effective amount of a compound of Formula (I).
This invention also constitutes a method for preventing and treating viral, fungal and bacterial infections including sepsis, in immunosuppressed and normal animals, including humans, which comprises administering to an animal in need thereof, an effective amount of a compound of Formula (I).

Detailed Description of the Invention

The compounds of the invention are represented by structural formula (I)



(I)

wherein:

A_1 and A_2 independently from each other are $Z-(CH_2)_p-(NR^{11})_q-$, wherein

Z is a 4 - 10 membered mono- or bicyclic heterocyclic ring system containing up to four heteroatoms N, O, S in the ring in which at least one heteroatom is N, and wherein the ring is substituted or unsubstituted by one or two C_{1-4}alkyl, F, Cl, Br, I, C_{1-4} alkoxy, $(CH_2)_mR^{13}$, oxo, oxime, O-C_{1-4}alkyloxime, hydroxy, $N(R^{12})_2$, acylamino or aminoacyl groups, 8, 9, 10 membered monocyclic ring systems being excluded;

R^1, R^2, R^3, R^4 and R^{11} independently hydrogen, C_{1-4}alkylC(O)R^{13},

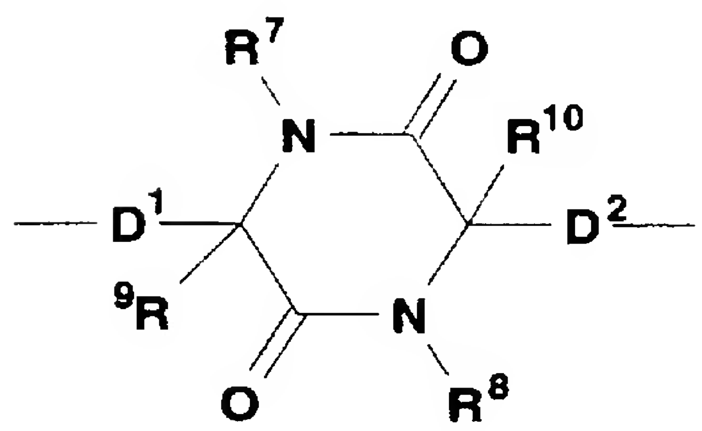
C_{1-4}alkyl or R^1, R^2, R^3, R^4 and R^{11} are benzyl which is optionally substituted by one or two C_{1-4}alkyl, C_{1-4}alkoxy, F, Cl, I, Br, OH, or $N(R^{12})_2$;

p is an integer from 0 to 4;

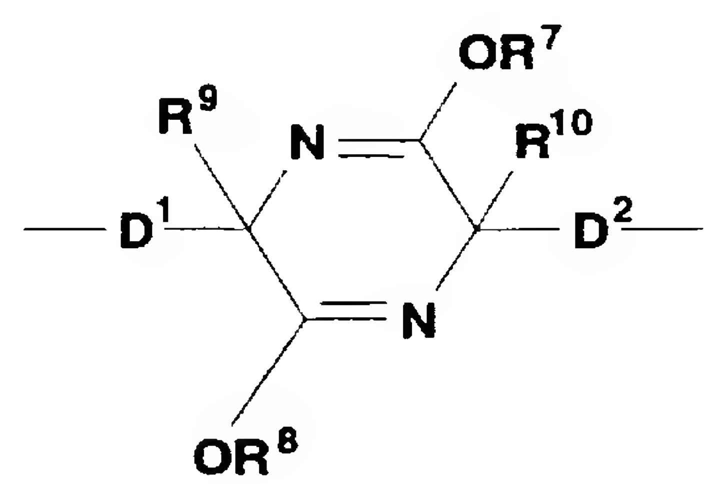
q, n and m are independently zero or one;

R^5 and R^6 are independently hydrogen, C_{1-4}-alkyl, C_{1-4}-alkyl-OH, C_{1-4}-alkyl-OCH_3, C_{1-4}-alkylaryl-OH, C_{1-4}-alkylaryl-OCH_3 or C_{1-4}-alkyl-COOH;

Q corresponds to structural formula (II) or (III)



(II)



(III)

wherein:

D_1 and D_2 are C_{1-8}-alkyl;

R^7, R^8, R^9 and R^{10} are independently hydrogen or C_{1-4}-alkyl;

R^{12} is independently hydrogen, C_1-C_4-alkyl or benzyl;

R^{13} is independently $-OR^{12}$, $-N(R^{12})_2$, $-SR^{12}$;

or a pharmaceutically acceptable salt thereof.

Z in the above Formula (I) denotes an optionally substituted pyrrolyl, isopyrrolyl, pyrazolyl, isoimidazolyl, triazolyl, iosxazolyl, oxazolyl, thiazolyl, isothiazolyl, oxadiazolyl, pyridinyl, pyridazinyl, pyrimidinyl, pyrazinyl, pyrrolidinyl, piperazinyl, triazinyl, morpholinyl, indolyl, indoleninyl,

isobenzazolyl, pyrindinyl, ioindazolyl, indoxazinyl, benzoxazolyl, quinolinyl, isoquinolinyl, cinnolinyl, quinazolinyl, naphthyridinyl, pyridopyridinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, quinoxalinyl, indolinyl, pyrrolidonyl, imidazolyl, imidazolidinyl, imidazolinyl, piperidyl, tetrazolyl, quinuclidinyl, azetidinyl, or purinyl.

Possible substituents for Z are C_{1-4}-alkyl, O-C_{1-4}-alkyl, C_{1-4}-alkyl-O-C_{1-4}-alkyl, oxo, oxime, O-C_{1-4}-alkyloxime, hydroxy, amino, N-C_{1-4}-alkylamino, N,N-di-C_{1-4}-alkylamino, CO, C_{1-4}-alkyl-CO and (C_{1-4}-alkyl)$_2$-NC(O)-.

R^5 and R^6 denote hydrogen, C_{1-4}-alkyl, C_{1-4}-alkyl-OH, C_{1-4}-alkyl-OCH_3, C_{1-4}-alkyl-(phenyl-OH), C_{1-4}-alkyl-(phenyl-OCH_3) and C_{1-4}-alkyl-(phenyl-COOH).

Preferred compounds are those wherein Z is optionally substituted pyridinyl, pyrimidinyl, pyrazinyl, pyridyl, pyridazinyl, quinolinyl, tetrahydroquinolinyl, azetidinyl, or pyrrolidinyl.

More preferred compounds are those wherein Z is optionally substituted 2-pyridinyl, 2-pyrimidinyl, 2-pyrazinyl, 2-pyrrolidon-5-yl, 2-pyridyl, 3-pyridyl, or pyrrolidinyl.

Alkyl groups may be straight or branched.

The compounds of the present invention may contain one or more asymmetric carbon atoms and may exist in racemic and optically active form. All the compounds and diastereomers are contemplated to be within the scope of the present invention.

Especially preferred compounds are:

ε,ε'-bis(picolinoyl-seryl)-[*cyclo*-(D-Lys-L-Lys)]

ε,ε'-bis(picolinoyl-seryl)-[*cyclo*-(D-Lys-D-Lys)]

ε,ε'-bis(picolinoyl-seryl)-[*cyclo*-(L-Lys-L-Lys)]

ε,ε'-bis(picolinoyl)-[*cyclo*-(Lys-Lys)]

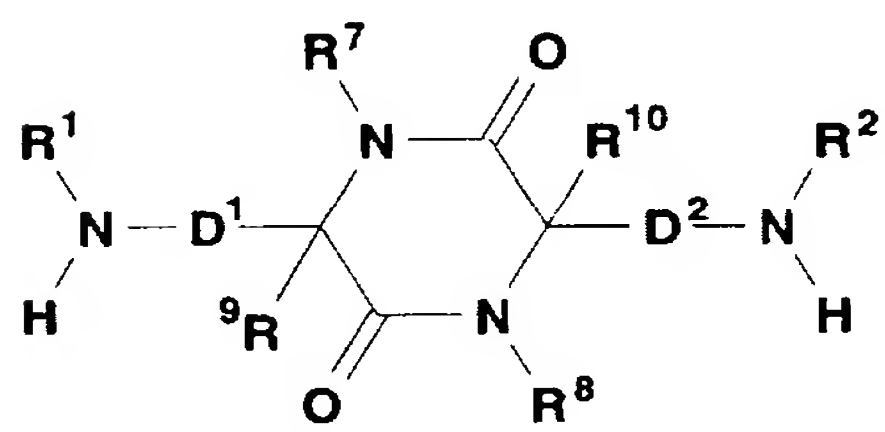
δ,δ'-bis(picolinoyl)-[*cyclo*-(Orn-Orn)]

γ,γ'-bis(picolinoyl)-[*cyclo*-(Dab-Dab)]

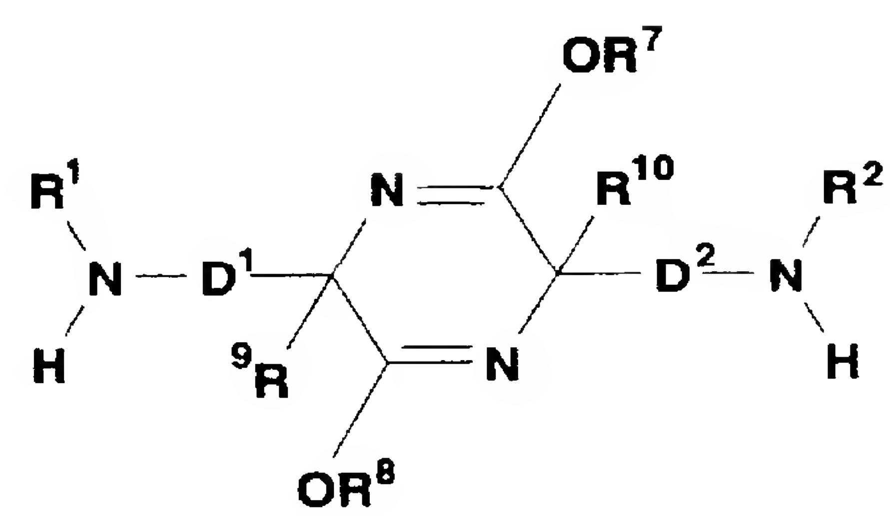
Methods of preparation

Compounds of formula (I) can be prepared as follows:

Suitable diamines of formula (IV) and (V) [definitions as in formulae (I), (II) and (III)]

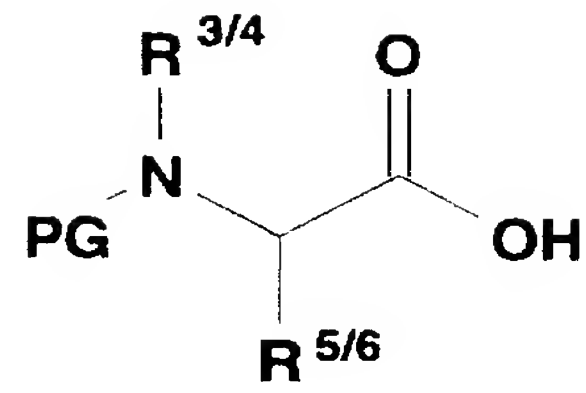


(IV)



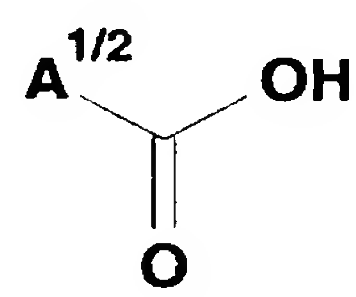
(V)

are reacted with two molar equivalents of an appropriate activated amino acid derivative of formula (VI), where the definitions are as in formula (I) and PG corresponds to suitable amino-protecting groups known in the art, e.g. t-butyloxycarbonyl, benzyloxycarbonyl, 9-fluorenylmethoxycarbonyl, etc. Where the amino acid side chains R^5 and R^6 contain hydroxyl or carboxyl groups, these are protected in the form of ethers or esters, chosen in such a way as to permit selective removal of the amino protecting group PG without regenerating the hydroxyl or carboxyl functions. A suitable combination of protecting groups would be when PG corresponds to benzyloxycarbonyl and the amino side chain hydroxyl or carboxyl is blocked in the form of a t-butyl ether or ester.



(VI)

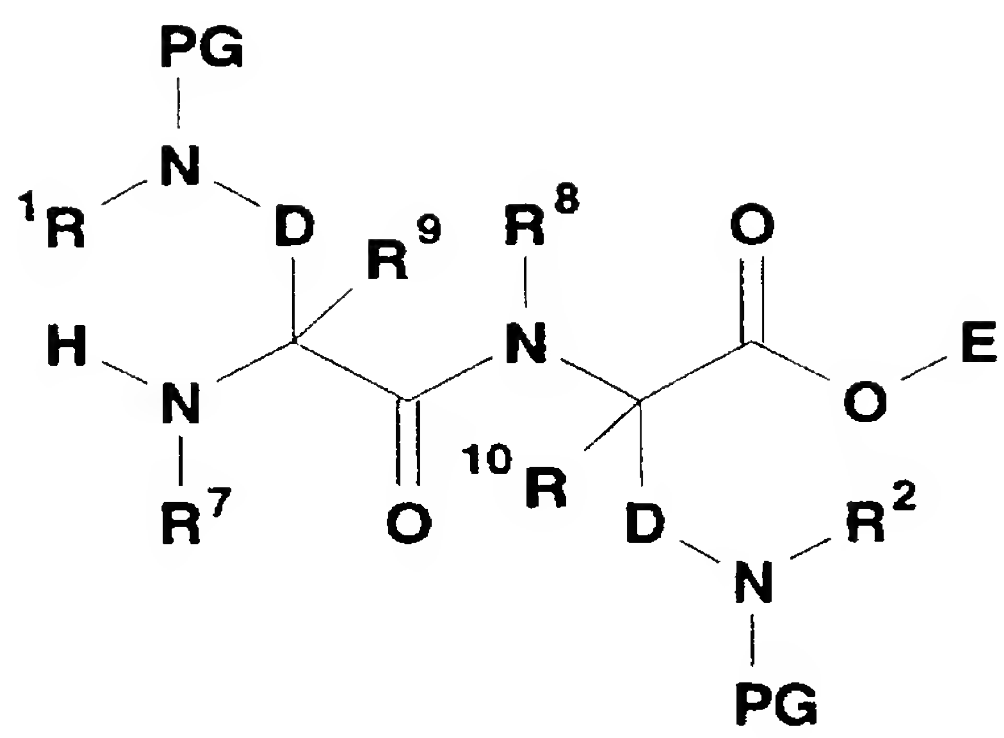
After removal of the protecting group PG (e.g. by hydrogenolysis in the case where PG corresponds to benzyloxycarbonyl), further acylation of the resulting diamine with two molar equivalents of an appropriately activated carboxylic acid derivative of formula (VII) is performed, followed by removal of the semi-permanent hydroxyl or carboxyl protecting groups (if present) in R^5 and R^6 (e.g. by acidolysis with trifluoroacetic acid in case of t-butyl ether/ester).



(VII)

in the case where n and m in formula (I) are zero, diamines of formula (IV) and (V) are reacted directly with appropriately activated carboxylic acid derivatives of formula (VII).

Diketopiperazine diamines of structure (IV) can be prepared by cyclisation of the corresponding dipeptidyl precursors of structure (VIII), in which the definitions are as in formulae (I), (II) and (III). Additionally, PG in structure (VIII) stands for suitable amino-protecting groups.



(VIII)

Cyclisation through intramolecular ester amminolysis may be acid- or base-catalysed or may be induced simply by heating a solution of compounds of structure (VIII) in some inert solvent. The ester portion E in structure (VIII) may correspond e.g. to methyl, ethyl, benzyl, N-hydroxysuccinimidyl, etc. After removal of protecting groups PG, compounds of structural formula (IV) are thus obtained, which in cases where R^7 and R^8 are hydrogen may be converted to the imino ether compounds of structure (V) e.g. through the action of trialkyl oxonium tetrafluoroborates.

In order to use a compound of the Formula (I) or a pharmaceutically acceptable salt thereof for the treatment of humans and other mammals it is normally formulated in accordance with pharmaceutical practice as a pharmaceutical composition.

According to a still further feature of the present invention there are provided pharmaceutical compositions comprising as active ingredient one or more compounds of Formula (I) as herein before defined or physiologically compatible salts thereof, in association with a pharmaceutical carrier or excipient. The compositions according to the invention may be presented for example, in a form suitable for oral, nasal, parenteral or rectal administration.

As used herein, the term "pharmaceutical" includes veterinary applications of the invention. These peptides may be encapsulated, tableted or prepared in an emulsion or syrup for oral administration. Pharmaceutically acceptable solid or liquid carriers

may be added to enhance or stabilize the composition, or to facilitate preparation of the composition. Liquid carriers include syrup, peanut oil, olive oil, glycerin, saline and water. Solid carriers include starch, lactose, calcium sulfate dihydrate, terra alba, magnesium stearate or stearic acid, talc, pectin, acacia, agar or gelatin. The carrier may also include a sustained release material such as glyceryl monostearate or glyceryl distearate, alone or with a wax. The amount of solid carrier varies, but, preferably will be between about 20 mg to about 1 g per dosage unit. The pharmaceutical preparations are made following the conventional techniques of pharmacy involving milling, mixing and filling for hard gelatin capsule forms. Capsules containing one or several active ingredients may be produced, for example, by mixing the active ingredients with inert carriers, such as lactose or sorbitol, and filling the mixture into gelatin capsules. Organ specific carrier systems may also be used.

Alternately pharmaceutical compositions of the peptides of this invention or derivatives thereof, may be formulated as solutions of lyophilized powders for parenteral administration. Powders may be reconstituted by addition of a suitable diluent or other pharmaceutically acceptable carrier prior to use. The liquid formulation is generally a buffered, isotonic, aqueous solution. Examples of suitable diluents are normal isotonic saline solution, standard 5% dextrose in water or buffered sodium or ammonium acetate solution. Such formulation is especially suitable for parenteral administration, but may also be used for oral administration and contained in a metered dose inhaler or nebulizer for insufflation. It may be desirable to add excipients such as polyvinylpyrrolidone, gelatin, hydroxycellulose, acacia, polyethylene glycol, mannitol, sodium chloride or sodium citrate.

For rectal administration, a pulverized powder of the peptides of this invention may be combined with excipients such as cocoa butter, glycerin, gelatin or polyethylene glycols and molded into a suppository. The pulverized powders may also be compounded with oily preparation, gel, cream or emulsion, buffered or unbuffered, and administered through a transdermal patch.

Nasal sprays may be formulated similarly in aqueous solution and packed into spray containers either with an aerosol propellant or provided with means for manual compression.

Dosage units containing the compounds of this invention preferably contain 0.05-50 mg, for example 0.05-5 mg of the compound of Formula (I) or of the salt thereof.

According to a still further feature of the present invention there is provided a method of stimulation of myelopoiesis which comprises administering an effective amount of a pharmaceutical composition as hereinbefore defined to a subject. No unacceptable toxicological effects are expected when compounds of the invention are administered in accordance with the present invention.

The biological activity of the compounds of Formula (I) is demonstrated by the following tests.

Induction of Hematopoietic Synergistic Activity in Stromal Cells

The murine bone marrow derived from stromal cell line C6.4 is grown in 12 well pates in RPMI 1640 with 10% FBS. Upon reaching confluence, the C6.4 cells are washed and the media exchanged with fresh RPMI 1640 without FBS. Confluent cell layers of murine C6.4 cells are treated with compound. Cell free supernatants are collected 18 hours later. Supernatants are fractionated with a Centricon-30 molecular weight cut-off membrane. C6.4 cell hematopoietic synergistic factor (HSF) activity is measured in a murine CFU-C assay.

CFU-C Assay

Bone marrow cells are obtained from C57B1/6 female mice and suspended in RPMI 1640 with 10% FBS. Bone marrow cells (7.5E+4 cells/mL) are cultured with sub optimal levels of CFU plus dilutions of test C6.4 cell 30K-E supernatants from above in a standard murine soft agar CFU-C assay. Cell aggregates >50 cells are counted as colonies. The number of agar colonies counted is proportional to the amount of HSF present within the C6.4 bone marrow stromal line supernatant.

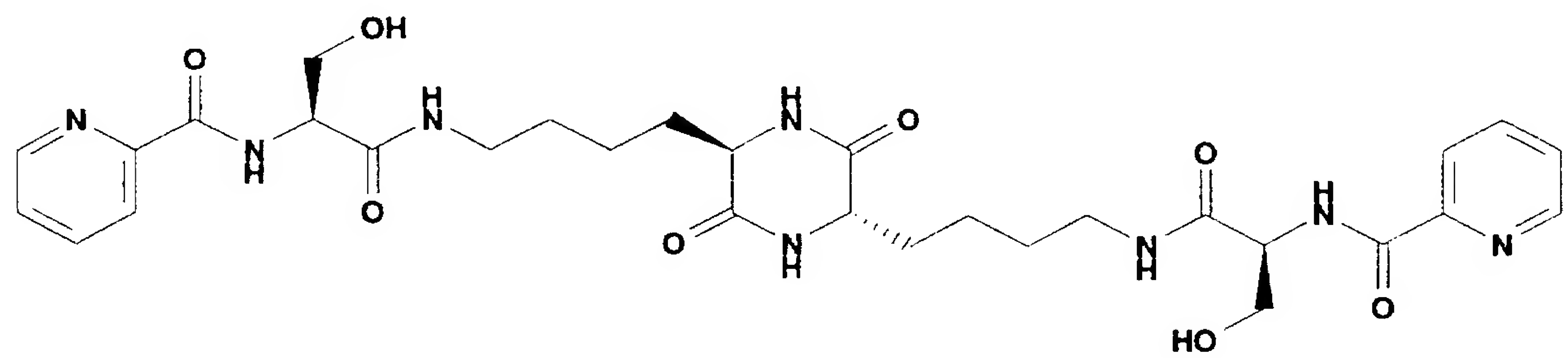
Effector Cell Function Assay

Female C57B1 mice are administered test compound PO daily for 8 days. Resident peritoneal exudate cells (PEC) utilized *ex vivo* from treated or untreated mice are harvested with cold calcium and magnesium-free DPBS supplemented with heparin and antibiotics within 2-4 hours following the last injection. Adherent PEM populations are prepared by incubating standardized PEC suspensions in microtiter dishes for 2 hours at 37 °C (5% CO_2) and removing nonadherent cells by washing the wells with warm buffer.

The superoxide dismutase-inhibitable (SOD) superoxide released by effector cells in response to a *in vitro* stimulation by phorbol myristate acetate (PMA) (100-200 nM) or pre-opsonized (autologous sera) live *C. albicans* (E:T = 1:10) are quantitated in a microtiter ferricytochrome *c* reduction assay. The assay is performed in the presence of 1% gelatin/HBSS and 80 µM ferricytochrome *c* in a total volume of 200 µL/well. The nmoles of cytochrome *c* reduced /well is calculated from spectrophotometric readings (550 nm) taken following a 1 hour incubation at 37 °C (5% CO_2). The amount of SOD-inhibitable cytochrome *c* reduced is determined by the inclusion of wells containing SOD (200 U/well). Baseline superoxide release is determined in the absence of stimuli. Experimental data are expressed as a percentage of the control group.

Examples

Example 1: Preparation of ε,ε'-bis(picolinoyl-seryl)-[*cyclo*-(D-Lys-L-Lys)]



a) Fmoc-D-Lys(Boc)-L-Lys(Z)-OMe

Fmoc-D-Lys(Boc)-OH (998 mg, 2.13 mmol) and NMM (0.23 mL, 2.13 mmol) were dissolved in THF (25 mL) and the solution was cooled to -15 °C. $Bu^{i}OCOCl$ (0.28 mL, 2.13 mmol) was then added. After 5 min a pre-cooled solution of H-L-Lys(Z)-OMe.HCl (706 mg, 2.13 mmol) and NMM (0.23 mL) in THF (25 mL) was added. The entire mixture was stirred and was allowed to reach room temperature. After 2 h precipitated NMM.HCl was filtered off and the filtrate evaporated. The residue was redissolved in CH_2Cl_2 (70 mL), extracted successively with 5 % aq $NaHCO_3$ and 10 % aq citric acid (2 x 25 mL each), dried over $MgSO_4$, filtered and evaporated to dryness. The title compound was obtained as a white powder (1.43 g, 90.1 %). TLC: R_F = 0.66 (85:10:5 $CHCl_3$/MeOH/AcOH). FAB-MS: $[M + H]^+$ =745.6, $[(M\text{-}Boc) + H]^+$ = 645.4; $C_{41}H_{52}N_4O_9$ requires 744.88.

b) *cyclo*-[D-Lys(Boc)-L-Lys(Z)]

Fmoc-D-Lys(Boc)-L-Lys(Z)-OMe (1.0 g, 1.34 mmol) was dissolved in 50 % Et_2NH/CH_2Cl_2 (50 mL) and the mixture was stirred during 2 d. The resulting suspension was evaporated, co-evaporated several times with PhMe and taken to dryness under high vacuum. The residue of H-D-Lys(Boc)-L-Lys(Z)-OMe (TLC:

R_f = 0.16, 85:10:5 $CHCl_3$/MeOH/AcOH) was resuspended in EtOAc (50 mL) and heated under reflux for 18 h. After this time the conversion to the diketopiperazine was complete as evidenced by TLC (R_F = 0.58 only). The cooled solution was evaporated to dryness. The residue was triturated with 10 % aq citric acid (50 mL), filtered and washed on the sinter with H_2O. After drying, the crude product was ground in Et_2O, filtered and dried to afford the pure title compound (406 mg, 61.7 %). FAB-MS: $[M + H]^+$ = 491.4, $[(M\text{-Boc}) + H]^+$ = 391.3; $C_{25}H_{38}N_4O_6$ requires 490.60.

c) ε,ε'-bis(picolinoyl-seryl)-[*cyclo*-(D-Lys-L-Lys)]

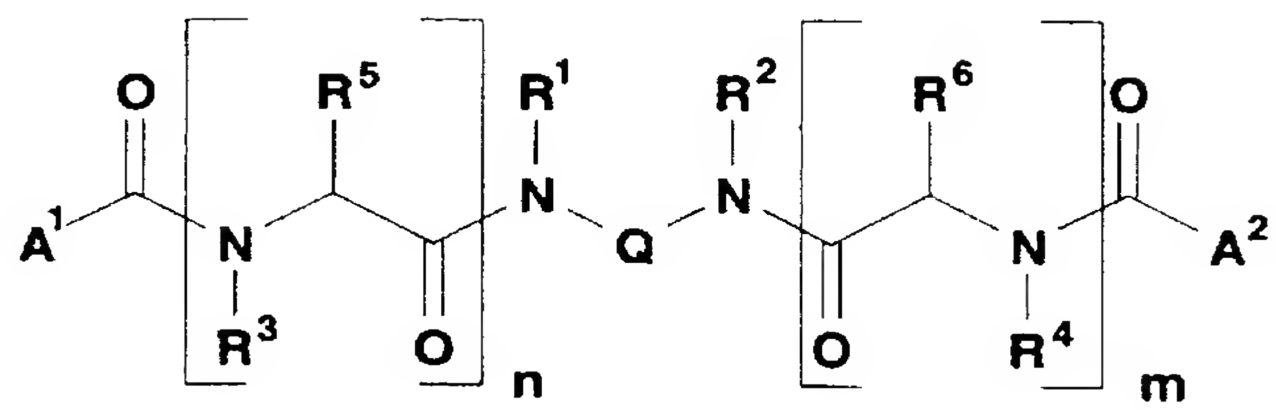
cyclo-[D-Lys(Boc)-L-Lys(Z)] (100 mg, 0.2 mmol) was dissolved in 1 M Me_3SiBr, 1 M PhSMe in CF_3COOH (20 mL) with ice-bath cooling. The mixture was stirred under N_2 and with cooling for 45 min. The cooling bath was then removed and stirring was continued for 30 min. H_2O (0.3 mL) was added and the mixture was evaporated. The residue was triturated with Et_2O and the precipitated product was collected by centrifugation. It was washed twice more with Et_2O and dried.

The resulting *cyclo*-[D-Lys-L-Lys] was suspended in DMF (25 mL) and added to a pre-activated (5 min) solution of Z-Ser(Bu^t)-OH (153 mg, 0.4 mmol), PyBOP (208 mg, 0.4 mmol), HOBt (54 mg, 0.4 mmol) and NMM (0.13 mL, 1.2 mmol) in DMF (5 mL). The mixture was stirred for 2 h. The resulting clear solution was evaporated and treated with 5 % aq $NaHCO_3$. The precipitated oil was extracted into CH_2Cl_2. The extract was washed successively with 10 % aq citric acid and 2 M aq NaCl. The organic layer was dried over $MgSO_4$, filtered and evaporated. The intermediate ε,ε'-bis[Z-Ser(Bu^t)]-[*cyclo*-(D-Lys-L-Lys)] was obtained as a discoloured oil. TLC: R_F = 0.62 (85:10:5 $CHCl_3$/MeOH/AcOH) and was used directly in the next reaction step.

ε,ε'-bis[Z-Ser(Bu^t)]-[*cyclo*-(D-Lys-L-Lys)] was redissolved in MeOH (50 mL) and was hydrogenolysed with 10 % Pd(C) catalyst (50 mg) for 90 min. The catalyst was then removed by filtration and the filtrate was evaporated. The oily residue was redissolved in DMF and added to a pre-activated (with PyBOP, HOBt and NMM as above; 5 min) solution of picolinic acid (49 mg, 0.4 mmol) in DMF (15 mL). After overnight reaction DMF was removed under vacuum. The residue was worked up as the previous intermediate except that no extraction with aqueous citric acid was performed. The intermediate ε,ε'-bis[picolinyl-Ser(Bu^t)]-[*cyclo*-(D-Lys-L-Lys)] (discoloured oil, TLC $R_F = 0.56$) was redissolved in 2 % aq CF_3COOH (25 mL) and the solution was stirred for 90 min. It was then evaporated and the residue was treated with Et_2O. The precipitated material was collected by centrifugation and drying to afford the crude title compound (16 mg, 12.0 %). This was dissolved in 4 mL 0.1 % aq CF_3COOH and chromatographed on a RP-HPLC column (Vydac 218TP1022) at 9 mL/min using a gradient from 20 to 40 % MeCN in 0.1 % aq CF_3COOH over 60 min. The eluant was monitored at 230 nm; appropriate peak fractions were collected, pooled and lyophilised to provide the pure title compound (3.0 mg). Anal. RP-HPLC: $t_R = 15.7$ min, purity > 98 % (Vydac 218TP54, 1 mL/min, 20 to 50 % MeCN in 0.1 % aq CF_3COOH over 20 min, $\lambda = 215$ nm). FAB-MS: $[M + H]^+ = 641.3$, $[M + Na]^+ = 663.2$; $C_{30}H_{40}N_8O_8$ requires 640.69.

We claim:

1. Compounds having hemoregulatory activity of the formula



(I)

wherein:

A_1 and A_2 independently from each other are Z-$(CH_2)_p$-$(NR^{11})_q$-, wherein

Z is a 4 - 10 membered mono- or bicyclic heterocyclic ring system containing up to four heteroatoms N, O, S in the ring in which at least one heteroatom is N, and wherein the ring is substituted or unsubstituted by one or two C_{1-4}alkyl, F, Cl, Br, I, C_{1-4} alkoxy, $(CH_2)_mR^{13}$, oxo, oxime, O-C_{1-4}alkyloxime, hydroxy, $N(R^{12})_2$, acylamino or aminoacyl groups, 8, 9, 10 membered monocyclic ring systems being excluded;

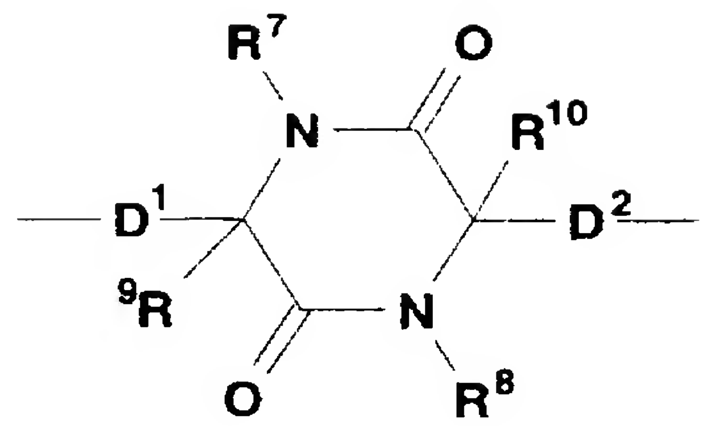
R^1, R^2, R^3, R^4 and R^{11} independently hydrogen, C_{1-4}alkylC(O)R^{13}, C_{1-4}alkyl or R^1, R^2, R^3, R^4 and R^{11} are benzyl which is optionally substituted by one or two C_{1-4}alkyl, C_{1-4}alkoxy, F, Cl, I, Br, OH, or $N(R^{12})_2$;

p is an integer from 0 to 4;

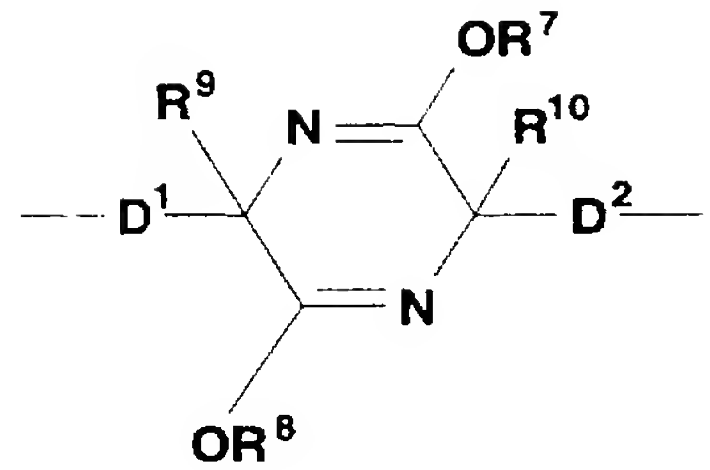
q, n and m are independently zero or one;

R^5 and R^6 are independently hydrogen, C_{1-4}-alkyl, C_{1-4}-alkyl-OH, C_{1-4}-alkyl-OCH_3, C_{1-4}-alkylaryl-OH, C_{1-4}-alkylaryl-OCH_3 or C_{1-4}-alkyl-COOH;

Q corresponds to structural formula (II) or (III)



(II)



(III)

wherein:

D_1 and D_2 are C_{1-8}-alkyl;

R^7, R^8, R^9 and R^{10} are independently hydrogen or C_{1-4}-alkyl;

R^{12} is independently hydrogen, C_1-C_4-alkyl or benzyl;

R^{13} is independently -OR^{12}, -$N(R^{12})_2$, - SR^{12};

or a pharmaceutically acceptable salt thereof.

2. Compounds according to claim 1, wherein Z denotes an optionally substituted pyrrolyl, isopyrrolyl, pyrazolyl, isoimidazolyl, triazolyl, iosxazolyl, oxazolyl, thiazolyl, isothiazolyl, oxadiazolyl, pyridinyl, pyridazinyl, pyrimidinyl, pyrazinyl, pyrrolidinyl, piperazinyl, triazinyl, morpholinyl, indolyl, indoleninyl, isobenzazolyl, pyrindinyl, ioindazolyl, indoxazinyl, benzoxazolyl, quinolinyl, isoquinolinyl, cinnolinyl, quinazolinyl, naphthyridinyl, pyridopyridinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, quinoxalinyl, indolinyl, pyrrolidonyl, imidazolyl, imidazolidinyl, imidazolinyl, piperidyl, tetrazolyl, quinuclidinyl, azetidinyl, or purinyl.

3. Compounds according to claim 2, wherein Z denotes an optionally substituted 2-pyridinyl, 2-pyrimidinyl, 2-pyrazinyl, 2-pyrrolidon-5-yl, 2-pyridyl, 3-pyridyl, or pyrrolidinyl.

4. Compounds according to claim 1, wherein Z is optionally mono-, poly- or mixed substituted by C_{1-4}-alkyl, O-C_{1-4}-alkyl, C_{1-4}-alkyl-O-C_{1-4}-alkyl, oxo, oxime, O-C_{1-4}-alkyloxime, hydroxy, amino, N-C_{1-4}-alkylamino, N,N-di-C_{1-4}-alkylamino, CO, C_{1-4}-alkyl-CO or $(C_{1-4}\text{-alkyl})_2$-NC(O)-.

5. Compounds according to any one of claims 1 to 3 wherein Z is optionally mono-, poly- or mixed substituted by methyl, ethyl, methoxy, methoxymethyl, oxo, oxime, hydroxy, amino, ethylamino or dimethylamino.

6. Compounds according to any one of claims 1 to 5 wherein R^1, R^2, R^3, R^4 and R^{11} independently from each other denote hydrogen, methyl, ethyl, propyl, butyl, C_{1-4}-alkylcarboxylic acid or C_{2-4}-alkylhydroxy.

7. Compounds according to any one of claims 1 to 6 wherein R^5 and R^6 denote hydrogen, C_{1-4}-alkyl, C_{1-4}-alkyl-OH, C_{1-4}-alkyl-OCH_3, C_{1-4}-alkyl-(phenyl-OH), C_{1-4}-alkyl-(phenyl-OCH_3) or C_{1-4}-alkyl-(phenyl-COOH).

8. Compounds according to any one of claims 1 to 7 wherein R^7, R^8, R^9 and R^{10} are independently hydrogen, methyl, ethyl, propyl or butyl.

9. A compound of Claim 1 which is chosen from the group consisting of:

ε,ε'-bis(picolinoyl-seryl)-[*cyclo*-(D-Lys-L-Lys)];

ε,ε'-bis(picolinoyl-seryl)-[*cyclo*-(D-Lys-D-Lys)];

ε,ε'-bis(picolinoyl-seryl)-[*cyclo*-(L-Lys-L-Lys)];

ε,ε'-bis(picolinoyl)-[*cyclo*-(Lys-Lys)];

δ,δ'-bis(picolinoyl)-[*cyclo*-(Orn-Orn)]; or

γ,γ'-bis(picolinoyl)-[*cyclo*-(Dab-Dab)].

10. Process for producing a compound as claimed in any one of claims 1 to 8, said process comprising

a) cyclising a suitably protected dipeptide through intramolecular ester amminolysis;

b) optionally converting the resulting diketopiperazine into the imminoether compound;

c) optionally reacting one equivalent of the resulting, suitably protected diamine with two equivalents of appropriate, suitably protected amino acids;

d) optionally removing the amino acid protecting groups

e) acylating the resulting diamine with heterocyclic acids.

f) optionally reducing and/or oxidizing any functional groups and/or removing any remaining protecting groups, and

g) optionally forming a pharmaceutically acceptable salt thereof.

11. A pharmaceutical composition comprising a compound according to any one of claims 1 to 8 and a pharmaceutically acceptable carrier.

12. A method of stimulating the myelopoietic system which comprises administering to a subject in need thereof, an effective amount to stimulate said myelopoietic system of a compound to any one of claims 1 to 8.

13. A method of preventing or treating viral, fungal and bacterial infections which comprises administering to a subject in need thereof, an effective amount of a compound of any one of claims 1 to 8.

14. A method of preventing or treating sepsis which comprises administering to a subject in need thereof, an effective amount of a compound of any one of claims 1 to 8.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/18245

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 38/07
US CL :530/330
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/330; 514/18

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS Online

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 4,499,081 (LAERUM) 12 February 1985, see entire document.	1-13

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date		
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search

06 FEBRUARY 1997

Date of mailing of the international search report

04 MAR 1997

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